PB89-236327

Genotoxic Assessment of Environmental Tobacco Smoke Using Bacterial Bioassays

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

1989

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1. REPORT NO.	2.	3. PB89-236327
EPA/600/J-89/014		

4. TITLE AND SUBTITLE
A GENOTOXIC ASSESSMENT OF ENVIRONMENTAL TOBACCO SMOKE USING BACTERIAL BIOASSAYS

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]Health Effects Research Laboratory, RTP/NC
[3]Research Triangle Institute, RTP, NC

10. PROGRAM ELEMENT NO.
ANNA1E

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory - RTP, NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/11

15. SUPPLEMENTARY NOTES
Presented: Mutation Research vol 222, pp. 81-99, 1989

16. ABSTRACT

Recently, the National Research Council in the U.S.A. stated that laboratory studies of environmental tobacco smoke (ETS) should be important in identifying ETS carcinogens and their concentrations in typical daily environments, and in understanding how these compounds contribute to ETS dose-response relationships. This paper demonstrates that integrated chemical and bacterial mutagenicity information can be used to identify ETS genotoxicants, monitor human exposure, and make comparative assessments. Approximately 1/3 of the ETS constituents for which there is quantitative analytical chemistry information also have associated genotoxicity information. For example, 11 of the quantitated compounds are animal carcinogens. Work presented in this paper demonstrates that both the nonparticle-bound semivolatile and the particulate-bound organic material contain bacterial mutagens. These ETS organics give an equivalent of ~86,000 revertants per cigarette. In addition, this article summarizes efforts to estimate ETS bacterial mutagenicity, to use bacterial tests for the monitoring of ETS-impacted indoor environments, and to use bacterial assays for the direct monitoring of human exposure.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	21
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA/600/J-89/014

Mutation Research, 222 (1989) 81–99
Elsevier

MTR 02102

A genotoxic assessment of environmental tobacco smoke using bacterial bioassays

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(Received 25 June 1987)
(Revision received 12 October 1987)
(Accepted 16 October 1987)

Keywords: Environmental tobacco smoke; *Salmonella typhimurium*; Indoor environments; Chemistry; Semi-volatiles; Monitoring

Summary

Recently, the National Research Council in the U.S.A. stated that laboratory studies of environmental tobacco smoke (ETS) should be important in identifying ETS carcinogens, their concentrations in typical daily environments, and in understanding how these compounds contribute to ETS dose–response relationships. This paper demonstrates that integrated chemical and bacterial mutagenicity information can be used to identify ETS genotoxicants, monitor human exposure, and make comparative assessments. Approximately 1/3 of the ETS constituents for which there is quantitative analytical chemistry information also have associated genotoxicity information. For example, 11 of the quantitated compounds are animal carcinogens. Work presented in this paper demonstrates that both the nonparticle-bound semi-volatile and the particulate-bound organic material contain bacterial mutagens. These ETS organics give an equivalent of ~ 86 000 revertants per cigarette. In addition, this article summarized efforts to estimate ETS bacterial mutagenicity, to use bacterial tests for the monitoring of ETS-impacted indoor environments, and to use bacterial assays for the direct monitoring of human exposure.

Environmental tobacco smoke (ETS) is the total tobacco smoke found in an environment and includes both sidestream cigarette smoke and the exhaled tobacco smoke of the smokers within the environment. The first suggestion that environmental tobacco smoke (ETS) could have detrimental health effects was a medical case report published by Rosen and Levy in 1950. This report concluded that an infant's severe astmatic symptoms were directly related to the mother's smoking of tobacco products. It was not until 31 years later that the results from epidemiological studies of passive smoking and lung cancer were available. Three studies were published in 1981. Based on a population of 91 540 nonsmoking Japanese housewives, Hirayama (1981) reported that the wives of heavy smokers (> 20 cigarettes/day) had 2.4 times the risk of developing lung cancer as the nonsmoking wives of nonsmokers. Trichopoulos et al. (1981) reported a slightly larger risk for nonsmoking Greek women whose husbands smoked

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more than 20 cigarettes per day. Although Garfinkel (1981) found a small (10–30%) increase in mortality associated with passive smoking, his study of 469000 nonsmokers did not detect a statistically significant increase in risk of developing lung cancer. Since that time, a number of other epidemiologic studies have emerged (National Research Council, 1986). The National Research Council (1986) concluded that the summary estimate of increased risk of lung cancer ranges from 10% to 34%. They also concluded that although bias may contribute to the results, the best estimate at present for increased adjusted risk of lung cancer to nonsmokers due to passive exposure is approximately 25%. In identifying needed scientific information, the National Research Council (1986) stated: 'Laboratory studies should be important in determining the carcinogenic constituents of ETS and their concentrations in typical daily environments and in facilitating understanding of possible dose–responsive relationships.' The purpose of this paper is to partially fulfill this need by integrating chemical and bacterial genetic bioassay information concerning sidestream and indoor air (IA) tobacco smoke.

Materials and methods

Integrating known chemical and genotoxicity information for sidestream cigarette smoke

Although it is expected that most of the compounds found in mainstream tobacco smoke (MS) would also be found in sidestream smoke (SS), the purpose of this effort was to extract from the literature compounds identified specifically in SS and to associate this information with presently available genotoxicity information. As part of an earlier effort to identify airborne compounds and genotoxicity information (Graedel et al., 1986), confirmed compounds found in tobacco smoke and indoor air were cataloged. This summary provided the baseline information for this tabulation. In addition, an on-line *Chemical Abstracts* search was used to locate relevant papers published since January, 1983. The compounds identified in these papers were summarized, and the MS, SS, and/or indoor air (IA) concentrations were tabulated. Where available, the bioassay summary information as reported by Graedel et al. (1986) was paired with the identified chemicals. All computer literature searches were kindly supplied by the Resource Information Center, U.S. Environmental Protection Agency, Research Triangle Park, NC (U.S.A.) via Ms. Libby Smith. Although the search periods covered a time period extending to December 1986, the information is not meant to be exhaustive but representative of the available literature.

Methods for determining the Salmonella mutagenicity of organic extracts from sidestream tobacco smoke particles

After being lit, a generic U.S. brand of filter cigarettes was allowed to burn within a 0.04-m^3 Plexiglass® chamber into which filtered air was allowed to enter. The generated SS was continuously exhausted at a rate of 0.03 m^3/min and collected on a 142-mm Teflon®-coated glass-fiber filter. Sample preparation was done in the manner reported by Morin et al. (1987). The method can be summarized as follows. All filter samples were extracted using 2 15-min sonications (Constant Temperature, Sonicor® waterbath sonicator, Bay Shore, NY) using either dichloromethane, methanol, or acetone as the solvent. Extracted samples were concentrated to 5–10 ml using rotatory evaporation. Samples were then transferred into 15-ml volumetric tubes and concentrated to 1–2 ml using dry nitrogen. After adding 15 ml of dimethyl sulfoxide (DMSO), the remaining solvent was removed using the nitrogen purge method. Negative controls were prepared in the same manner using filters not containing ETS particles. The solvent-exchanged samples were tested using the *Salmonella typhimurium* plate incorporation assay as previously described (Ames et al., 1975; Claxton et al., 1987).

Direct comparisons of mainstream and sidestream tobacco smoke bacterial mutagenicity

In order to compare the total genotoxic potential of MS and SS it was necessary to bioassay the particle-bound, semi-volatile, and volatile compounds emitted. In order to accomplish this, separate trapping trains for MS and SS were used. Each train consisted of an ethanol bubbler solvent trap, a sand trap, and a liquid-nitrogen cold trap in sequence (Monteith et al., 1986). Cigarette sam-

ples were generated using a smoking machine. The standard conditions were 1 puff/min for each of the 30 cigarettes (1R3 Kentucky Reference), a puff duration of 2 s, and a puff volume of 35 ml. Mainstream smoke was pulled directly into the MS trapping train. In order to collect the SS, the cigarette smoking machine was enclosed within a Tedlar® bag which had a total volume of 0.12 m^3. Air from the bag was continuously drawn into the SS trapping trains. The ethanol bubbler collected primarily semi-volatile compounds, the sand trap collected primarily particulate matter, and the cold trap collected any remaining organic compounds. The sand trap and semi-volatile concentrates were bioassayed in sealed dram vials using a modified version of the preincubation protocol of Yahagi et al. (1975). Preincubation was for 15 min at 37°C while vials were shaken by a rotary shaker at 60 rpm. Tests were done using 500 µl/plate of 5% Aroclor-1254-induced male Syrian hamster liver homogenate. Volatiles from the cold trap were transferred as a gas to Tedlar® bags containing standard bioassay plates. All other aspects of the test were conducted as described by Ames et al. (1975).

Results and discussion

When one evaluates the hazard of a complex organic emission such as ETS, there are multiple and varied approaches that one may use. As stated in the introduction, several researchers have assessed ETS through epidemiological efforts (Hirayama, 1981; Trichopoulos et al., 1981; Garfinkel, 1981; National Research Council, 1986). Although such studies can demonstrate the statistical likelihood of increased risk associated with ETS, these studies were not designed to answer more specific questions concerning ETS and human health. For example, these studies do not identify which constituents within ETS emissions are carcinogens, mutagens, and other types of toxicants. Epidemiology, therefore, is unlikely to determine whether or not changes in tobacco type used, cigarette design, tar values, room ventilation rates, etc. will produce major changes in the health impact of ETS. When human epidemiological data are unavailable and unlikely to be available to answer such relevant issues, one can sometimes use whole animal studies (Stara and Kello, 1979). However, since whole animal studies for carcinogenesis and heritable mutations are expensive, lengthy processes, they too are unlikely to be useful for answering many relevant questions. Short-term test data, especially when associated with quantitative data on analytical chemistry, can be used to make comparative assessments, to identify genotoxicants, and to monitor human exposure.

After identification and quantification of the individual constituents of the mixture, the known toxicological properties of each constituent can be related to the source, for example ETS. The total potential impact of a source can then be estimated by summing the activity of the known constituents or by using some form of toxicological modeling or scaling.

Approximately 10% of the more than 3800 compounds found in MS have been identified in ETS. Table 1 lists over 100 constituents found in ETS and in MS and IA samples for which there is quantitative analytical information. The relative concentrations of the constituents vary according to whether they are measured as MS, SS, or IA components. The reasons (e.g., combustion temperature, oxygen levels, etc.) for differences in concentrations of organic components in MS and SS have been discussed previously (Sakuma et al., 1983, 1984; Baker, 1981, 1982; Klus and Kutin, 1982; IARC, 1968; National Research Council, 1986).

Table 2 lists the genotoxicity information (Graedel et al., 1986; Nesnow et al., 1987) for the compounds in Table 1. Approximately 1/3 of the compounds in Table 1 have associated genotoxicity information. Eleven of these compounds are animal carcinogens (Nesnow et al., 1987). Nineteen of the compounds are positive in at least 1 bioassay, and 10 are positive in the Salmonella bioassay (Graedel et al., 1986). This type of information provides a qualitative evaluation of potential human health impacts. For comparative risk analysis methods (Lewtas, 1985), the amount of each constituent can be associated with the bioassay activity of each constituent in order to provide a crude means of comparison between sources or components of interest. For example, using concentrations of constituents in SS and their known

TABLE 1

CONCENTRATIONS OF COMPOUNDS ASSOCIATED WITH MAINSTREAM AND SIDESTREAM TOBACCO SMOKE AND INDOOR AIR POLLUTED WITH TOBACCO SMOKE [a]

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Acetamide	MS	70.00	111.00	μg/cig	25
Acetamide	SS	86.00	156.00	μg/cig	25
Acetic acid	MS	333.00	809.00	μg/cig	23
Acetic acid	MS	272.00	475.00	μg/cig	25
Acetic acid	SS	1241.00	2187.00	μg/cig	23
Acetic acid	SS	695.00	1148.00	μg/cig	25
Acrolein	IA	0.90	1.30	ppm	2
Acrolein	IA	0.02	0.12	mg/m^3	3
Acrolein	IA	6.00	10.00	ppm	12
Acrolein	IA	0.01	0.19	mg/m^3	19
Acrolein	SS	50.00	70.00	ppm	2
Acrolein (gas only + people)	IA	130.00	190.00	$\mu g/m^3$	19
Acrolein (people absent)	IA	119.00	133.00	$\mu g/m^3$	19
Acrolein (people present)	IA	10.00	48.00	$\mu g/m^3$	19
Acrolein control air	IA	0.00	5.00	ppm	12
Aldehydes (gas only + people)	IA	1290.00	1350.00	$\mu g/m^3$	19
Aldehydes (generic)	IA	0.39	1.37	mg/m^3	19
Aldehydes (people absent)	IA	1100.00	1370.00	$\mu g/m^3$	19
Aldehydes (people present)	IA	391.00	622.00	$\mu g/m^3$	19
Alkoxyl radicals	MS	8.00×10^{15} spins/c			22
Alkoxyl radicals	SS	6.00×10^{6} spins/c			22
Ammonia	MS	79.40	131.00	μg/cig	9
Ammonia	MS	95.30	163.00	μg/g smoked	9
Ammonia (cigars)	MS	30.50	322.00	μg/g smoked	9
Ammonia (cigars)	MS	148.00	288.00	μg/product	9
Ammonia	SS	5.14	5.77	mg/cig.	9
Ammonia	SS	6.11	7.18	mg/g smoked	9
Ammonia (cigars)	SS	6.98	106.00	mg/cig	9
Ammonia (cigars)	SS	9.34	20.50	mg/g smoked	9
Anatabine	MS	2.40	20.10	μg/cig	24
Anatabine	SS	0.00	2.40	μg/cig	24
Anthanthrene	IA			Qual (ng/m^3)	1
Anthanthrene	IA	3.00		ng/m^3	15
Anthanthrene	MS	22.00		ng/cig	14
Anthanthrene	SS	39.00		ng/cig	14
Anthracene	IA			Qual (ng/m^3)	1
Anthracene	MS, P	23.60		ng/cig	16
Anthracene	MS, V	0.10		ng/cig	16
Anthracene	SS, P	670.00		ng/cig	16
Anthracene	SS, V	40.00		ng/cig	16
Benz[*a*]anthracene	IA			Qual (ng/m^3)	1
Benz[*a*]anthracene	MS, P	13.30		ng/cig	16
Benz[*a*]anthracene	MS, V	0.09		ng/cig	16
Benz[*a*]anthracene	SS, P	201.00		ng/cig	16
Benz[*a*]anthracene	SS, V	2.50		ng/cig	16

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Benz[*e*]acenaphthylene	IA			Qual (ng/m^3)	1
Benzene	IA	0.05	0.15	mg/m^3	3
Benzene (breath, non-smokers)	IA	2.50		$\mu g/m^3$	30
Benzene (breath, smokers)	IA	16.00		$\mu g/m^3$	30
Benzene (homes, non-smokers)	IA	4.40	9.20	$\mu g/cm^3$	30
Benzene (homes, smokers)	IA	4.80	16.00	$\mu g/m^3$	30
Benzo[*a*]fluorene	IA			Qual (ng/m^3)	1
Benzo[*a*]fluorene	IA	39.00		ng/m^3	15
Benzo[*a*]fluorene	MS	184.00		ng/cig	14
Benzo[*a*]fluorene	SS	751.00		ng/cig	14
Benzo[*a*]pyrene	IA			Qual (ng/m^3)	1
Benzo[*a*]pyrene	IA	7.10	21.70	ng/m^3	11
Benzo[*a*]pyrene	IA	6.20	144.00	ng/m^3	13
Benzo[*a*]pyrene	IA	22.00		ng/m^3	15
Benzo[*a*]pyrene	MS	44.00		ng/cig	14
Benzo[*a*]pyrene	MS, P	10.90		ng/cig	16
Benzo[*a*]pyrene	MS, V	0.08		ng/cig	16
Benzo[*a*]pyrene	SS	199.00		ng/cig	14
Benzo[*a*]pyrene	SS, P	103.00		ng/cig	16
Benzo[*a*]pyrene	SS, V	0.48		ng/cig	16
Benzo[*a*]pyrene control	IA	0.00	0.69	ng/m^3	11
Benzo[*b*]naphtho[2,1-d]thiophene	MS, P	2.80		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	MS, V	0.21		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	SS, P	50.00		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	SS, V	1.10		ng/cig	16
Benzo[*b/c*]fluorene	MS	69.00		ng/cig	14
Benzo[*b/c*]fluorene	SS	251.00		ng/cig	14
Benzo[*b/j/k*]fluoranthene	IA	35.00		ng/m^3	15
Benzo[*b/j/k*]fluoranthene	MS	49.00		ng/cig	14
Benzo[*b/j/k*]fluoranthene	SS	260.00		ng/cig	14
Benzo[*e*]fluorene	IA			Qual (ng/m^3)	1
Benzo[*e*]pyrene	IA			Qual (ng/m^3)	1
Benzo[*e*]pyrene	IA	18.00		ng/m^3	15
Benzo[*e*]pyrene	IA	3.30	23.40	ng/m^3	20
Benzo[*e*]pyrene	MS	25.00		ng/cig	14
Benzo[*e*]pyrene	MS, P	6.70		ng/cig	16
Benzo[*e*]pyrene	MS, V	0.13		ng/cig	16
Benzo[*e*]pyrene	SS	135.00		ng/cig	14
Benzo[*e*]pyrene	SS, P	75.00		ng/cig	16
Benzo[*e*]pyrene	SS, V	0.74		ng/cig	16
Benzo[*e*]pyrene control	IA	3.00	5.10	ng/m^3	20
Benzo[*ghi*]fluoranthene	IA			Qual (ng/m^3)	1
Benzo[*ghi*]perylene	IA			Qual (ng/m^3)	1
Benzo[*ghi*]perylene	IA	17.00		ng/m^3	15
Benzo[*ghi*]perylene	MS	39.00		ng/cig	14
Benzo[*ghi*]perylene	MS, P	7.10		ng/cig	16
Benzo[*ghi*]perylene	MS, V	0.09		ng/cig	16
Benzo[*ggi*]perylene	SS	98.00		ng/cig	14
Benzo[*ghi*]perylene	SS, P	41.00		ng/cig	16
Benzo[*ghi*]perylene	SS, V	0.62		ng/cig	16

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Benzofluoranthenes (b + j + k)	MS, P	20.50		ng/cig	16
Benzofluoranthenes (b + j + k)	MS, V	0.22		ng/cig	16
Benzofluoranthenes (b + j + k)	SS, P	196.00		ng/cig	16
Benzofluoranthenes (b + j + k)	SS, V	1.38		ng/cig	16
Benzoic acid	MS	14.00	28.00	μg/cig	23
Benzoic acid	SS	12.00	23.00	μg/cig	23
Benzoic acid, *m*-hydroxy-	MS	8.00	64.00	μg/cig	23
Benzoic acid, *m*-hydroxy-	SS	3.00	15.00	μg/cig	23
Benzonitrile	MS	5.00	6.00	μg/cig	25
Benzonitrile	SS	33.00	57.00	μg/cig	25
Bipyridyl, 2, 3′-	MS	9.90	27.40	μg/cig	24
Bipyridyl, 2, 3′-	SS	20.00	73.00	μg/cig	24
Bipyridyl, 5-methyl-2, 3′-	MS	6.60	14.70	μg/cig	24
Bipyridyl, 5-methyl-2, 3′-	SS	6.00	14.00	μg/cig	24
Butyrolactone, gamma-	SS	40.00	103.00	μg/cig	25
Butyrolactone, gamma-	MS	11.00	22.00	μg/cig	25
Carbon monoxide	IA	2.00	23.00	ppm	3
Carbon monoxide	IA	0.00	1.20	ppm	31
Carbon monoxide (gas only + people)	IA	23.00	26.00	ppm	19
Carbon monoxide (people absent)	IA	21.00	25.00	ppm	19
Carbon monoxide (people present)	IA	18.00	22.00	ppm	19
Carbon monoxide (people present)	IA	3.70	4.20	ppm	28
Carbon monoxide control	IA	0.00	15.00	ppm	3
Carbon monoxide control	IA	0.00	0.50	ppm	31
Carbon monoxide (artificial cond.)	IA	8.00	16.00	ppm	29
Carbon monoxide (natural conditions)	IA	9.00		ppm	29
Carbon, total	IA	207.00		$\mu g/m^3$	27
Carbon, elemental	IA	11.90		$\mu g/m^3$	27
Carbon, organic	IA	195.00		$\mu g/m^3$	27
Carboxyhemoglobin (blood, passive)	IA	0.55		%	28
Carboxyhemoglobin (blood, smoker)	IA	3.38		%	28
Carboxyhemoglobin (blood, no smoking)	IA	0.57		%	28
Catechol	MS	148.00	362.00	μg/cig	23
Catechol	SS	138.00	292.00	μg/cig	23
Catechol, 2-methyl-	MS	6.00	13.00	μg/cig	23
Catechol, 2-methyl-	SS	8.00	21.00	μg/cig	23
Catechol, 3-methyl-	MS	31.00	62.00	μg/cig	23
Catechol, 3-methyl-	SS	24.00	47.00	μg/cig	23
Catechol, 4-ethyl-	MS	27.00	102.00	μg/cig	23
Catechol, 4-ethyl-	SS	19.00	68.00	μg/cig	23
Catechol, 4-methyl-	SS	25.00	55.00	$\mu g/m^3$	23
Catechol, 4-methyl-	MS	29.00	80.00	μg/cig	23
Catechol, 4-vinyl-	MS	23.00	113.00	μg/cig	23
Catechol, 4-vinyl-	SS	7.00	40.00	μg/cig	23
Catechols (all catechols)	MS	25.00	328.00	μg/cig	4
Catechols (all catechols)	SS	88.00	212.00	μg/cig	4
Coronene	IA	0.50	1.20	ng/m^3	20
Coronene control	IA	1.00	2.80	ng/m^3	20

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Cotinine (plasma, non-smoker)	IA	1.40		ng/ml	28
Cotinine (plasma, passive smoker)	IA	2.10		ng/ml	28
Cotinine (plasma, smoker)	IA	52.40		ng/ml	28
Cresol, *m*-	MS	11.00	18.00	μg/cig	23
Cresol, *m*-	MS	17.00	26.00	μg/cig	25
Cresol, *m*-	SS	13.00	24.00	μg/cig	23
Cresol, *m*-	SS	18.00	34.00	μg/cig	25
Cresol, *o*-	MS	13.00	19.00	μg/cig	23
Cresol, *o*-	SS	14.00	24.00	μg/cig	23
Cresol, *p*-	MS	30.00	37.00	μg/cig	23
Cresol, *p*-	MS	32.00	47.00	μg/cig	25
Cresol, *p*-	SS	30.00	46.00	μg/cig	23
Cresol, *p*-	SS	45.00	62.00	μg/cig	25
Cyclopentenone, 2, 3-dimethyl-2-	MS	9.00	23.00	μg/cig	25
Cyclopentenone, 2, 3-dimethyl-2-	SS	21.00	39.00	μg/cig	25
Cyclopentenone, 2-	MS	21.00	27.00	μg/cig	25
Cyclopentenone, 2-	SS	70.00	103.00	μg/cig	25
Cyclopentenone, 2-*OH*-3-methyl-2-	MS	3.00	5.00	μg/cig	25
Cyclopentenone, 2-*OH*-3-methyl-2-	SS	24.00	30.00	μg/cig	25
Cyclopentenone, 2-methyl-2-	MS	17.00	22.00	μg/cig	25
Cyclopentenone, 2-methyl-2-	SS	49.00	95.00	μg/cig	25
Dibenz[*a*, *j*]anthracene	IA	6.00		ng/m^3	15
Dibenz[*a*, *j*]anthracene	MS	11.00		ng/cig	14
Dibenz[*a*, *j*]anthracene	SS	41.00		ng/cig	14
Ethylbenzene (breath, non-smokers)	IA	0.80		$\mu g/m^3$	30
Ethylbenzene (breath, smokers)	IA	2.60		$\mu g/m^3$	30
Ethylbenzene (homes, non-smokers)	IA	3.50	5.10	$\mu g/m^3$	30
Ethylbenzene (homes, smokers)	IA	3.50	8.30	$\mu g/m^3$	30
Ethylmethylenephenanthrene	IA			Qual (ng/m^3)	1
Fluoranthene	IA			Qual (ng/m^3)	1
Fluoranthene	IA	99.00		ng/m^3	15
Fluoranthene	MS	272.00		ng/cig	14
Fluoranthene	MS, P	61.30		ng/cig	16
Fluoranthene	SS	1255.00		ng/cig	14
Fluoranthene	SS, P	669.00		ng/cig	16
Fluoranthene	SS, V	16.90		ng/cig	16
Formaldehyde	IA	1.50	2.10	ppm	2
Formaldehyde	IA	0.10	0.16	ppm	28
Formaldehyde	SS	80.00	110.00	ppm	2
Formic acid	MS	210.00	478.00	μg/cig	23
Formic acid	SS	341.00	665.00	μg/cig	
Furaldehyde, 2-	MS	15.00	43.00	μg/cig	25
Furaldehyde, 2-	SS	113.00	290.00	μg/cig	25
Furaldehyde, 5-methyl-	MS	6.00	29.00	μg/cig	25
Furaldehyde, 5-methyl-	SS	20.00	127.00	μg/cig	25
Furfuryl alcohol	MS	18.00	65.00	μg/cig	25
Furfuryl alcohol	SS	73.00	283.00	μg/cig	25

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Furoic acid, 2-	MS	44.00	107.00	μg/cig	23
Furoic acid, 2-	SS	25.00	60.00	μg/cig	23
Glutaric acid	MS	10.00	58.00	μg/cig	23
Glutaric acid	SS	6.00	18.00	μg/cig	23
Glycolic acid	MS	37.00	126.00	μg/cig	23
Glycolic acid	SS	35.00	77.00	μg/cig	23
Guaiacol, 4-vinyl-	SS	24.00	32.00	μg/cig	23
Guaiacol, 4-vinyl-	MS	23.00	36.00	μg/cig	23
Guaiacol, 4-vinyl-	MS	16.00	30.00	μg/cig	25
Guaiacol, 4-vinyl-	SS	15.00	37.00	μg/cig	25
HCN	IA	0.01	0.08	mg/m^3	19
HCN (gas only + people)	IA	82.00	86.00	$\mu g/m^3$	19
HCN (people absent)	IA	50.00		$\mu g/m^3$	19
HCN (people present)	IA	10.00	14.00	$\mu g/m^3$	19
Hydrazine	MS	31.50		ng/cig	21
Hydrazine	SS	94.20		ng/cig	21
Hydroquinone	MS	114.00	300.00	μg/cig	23
Hydroquinone	SS	91.00	285.00	μg/cig	23
Hydroquinone, methyl-	MS	23.00	39.00	μg/cig	23
Hydroquinone, methyl-	SS	21.00	41.00	μg/cig	23
Hydroxypropionic acid, 3-	MS	2.00	31.00	μg/cig	23
Hydroxypropionic acid, 3-	SS	1.00	29.00	μg/cig	23
Indeno[1, 2, 3-*cd*]pyrene	IA			Qual (ng/m^3)	1
Indeno[1, 2, 3-*cd*]pyrene	MS, V	0.17		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	SS, P	51.00		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	SS, V	0.36		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	MS, P	8.10		ng/cig	16
Isoquinoline	MS	1.60	2.00	μg/cig	24
Isoquinoline	SS	5.00	8.00	μg/cig	24
Lactic acid	MS	63.00	174.00	μg/cig	23
Lactic acid	SS	45.00	123.00	μg/cig	23
Levulinic acid	MS	29.00	56.00	μg/cig	23
Levulinic acid	SS	25.00	49.00	μg/cig	23
Limonene	MS	15.00	49.00	μg/cig	25
Limonene	SS	63.00	397.00	μg/cig	25
Lutidine, 2, 4-	SS	35.00	315.00	μg/cig	6
Lutidine, 2, 6-	MS	1.40	33.00	μg/cig	6
Lutidine, 2, 6-	SS	1.40	33.00	μg/cig	6
Lutidine, 3, 5-	MS	0.00	17.00	μg/cig	6
Lutidine, 3, 5-	SS	22.00	251.00	μg/cig	6
Methylenephenanthrene, 4, 5-	IA			Qual (ng/m^3)	1
Methylnaphthalene, 1-	SS	30.00		μg/cig	26
Methylnaphthalene, 1-	MS	1.02		μg/cig	26
Methylnaphthalene, 2-	MS	1.21		μg/cig	26
Methylnaphthalene, 2-	SS	31.60		μg/cig	26

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Methylnitrosoamino-pyridyl-butanone	MS	46.00	240.00	ng/cig	8
Methylnitrosoamino-pyridyl-butanone	SS	201.00	540.00	ng/cig	8
Methylphenanthrene, 1-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 2-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 3-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 4/9-	IA			Qual (ng/m^3)	1
Myosmine	MS	13.10	33.00	μg/cig	24
Myosmine	SS	73.00	224.00	μg/cig	24
Naphthalene	MS	2.76		μg/cig	26
Naphthalene	SS	45.50		μg/cig	26
Neophytadiene	MS	66.00	232.00	μg/cig	25
Neophytadiene	SS	70.00	421.00	μg/cig	25
Nicotine	IA	25.00	1010.00	μg/m^3	3
Nicotine	IA	0.70	3.10	μg/m^3	17
Nicotine	IA	1.00	10.30	μg/m^3	18
Nicotine	IA	1.70	180.00	pg/m^2 min	32
Nicotine	MS	1720.00	3330.00	μg/cig	24
Nicotine	MS	1483.00	3149.00	μg/cig	25
Nicotine	SS	3210.00	5830.00	μg/cig	24
Nicotine	SS	2987.00	6588.00	μg/cig	25
Nicotine (gas only + people)	IA			Traces only	19
Nicotine (people absent)	IA	130.00		μg/m^3	19
Nicotine (people present)	IA	102.00		μg/m^3	19
Nicotine, office buildings	IA	1.70	180.00	pg/m^2 min	32
Nicotyrine	MS	4.20	20.20	μg/cig	24
Nicotyrine	MS	17.00	41.00	μg/cig	25
Nicotyrine	SS	49.00	211.00	μg/cig	24
Nicotyrine	SS	93.00	263.00	μg/cig	25
Nitrogen dioxide	IA	0.00	0.03	ppm	19
Nitrogen dioxide	IA	58.00		ppb	31
Nitrogen dioxide (gas only + people)	IA	0.01	0.03	ppm	19
Nitrogen dioxide (people absent)	IA	0.00		ppm	19
Nitrogen dioxide (people present)	IA	0.00		ppm	19
Nitrogen dioxide control	IA	27.00		ppb	31
Nitrogen oxide	IA	0.30	0.60	ppm	19
Nitrogen oxide	IA	0.00	9.00	ppb	31
Nitrogen oxide (gas only + people)	IA	0.31	0.40	ppm	19
Nitrogen oxide (people absent)	IA	0.48	0.59	ppm	19
Nitrogen oxide (people present)	IA	0.30	0.60	ppm	19
Nitrogen oxide control	IA	5.00		ppb	31
Nitrogen oxides (combined)	IA	59.00	218.00	ppb	12
Nitrosoamine, methylethyl-	MS	0.10	9.10	ng/cig	5
Nitrosoamine, methylethyl-	MS	0.00	1.80	ng/cig	7
Nitrosoamine, methylethyl-	SS	9.00	75.00	ng/cig	5
Nitrosoamine, methylethyl-	SS	0.00	27.00	ng/cig	7
Nitrosoanabasine, *N'*-	SS	15.00	40.00	ng/cig	8
Nitrosoanatidine, *N'*-	MS	82.00	167.00	ng/cig	8
Nitrosoanatidine, *N'*-	SS	61.00	220.00	ng/cig	8

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Nitrosodiethylamine	MS	0.00	4.80	ng/cig	5
Nitrosodiethylamine	SS	8.00	73.00	ng/cig	5
Nitrosodiethylamine, *N*-	MS	1.80	4.80	ng/cig	10
Nitrosodiethylamine, *N*-	SS	8.20	73.00	ng/cig	10
Nitrosodiethylamine (artificial)	IA	0.00	0.01	ng/l	29
Nitrosodiethylamine (natural cond.)	IA	0.00	0.20	ng/l	29
Nitrosodimethylamine	MS	1.70	97.00	ng/cig	5
Nitrosodimethylamine	MS	0.00	27.00	ng/cig	7
Nitrosodimethylamine	SS	680.00	1770.00	ng/cig	5
Nitrosodimethylamine	SS	143.00	415.00	ng/cig	7
Nitrosodimethylamine	SS	460.00	1880.00	ng/cig	8
Nitrosodimethylamine, *N*-	MS	1.70	97.00	ng/cig	10
Nitrosodimethylamine, *N*-	SS	680.00	1040.00	ng/cig	10
Nitrosodimethylamine (artificial)	IA	0.02	0.15	ng/l	29
Nitrosodimethylamine (natural cond.)	IA	0.00	0.70	ng/l	29
Nitrosoethylmethylamine, *N*-	MS	0.00	9.10	ng/cig	10
Nitrosoethylmethylamine, *N*-	SS	9.40	30.00	ng/cig	10
Nitrosonornicotine	MS	81.00	390.00	ng/cig	8
Nitrosonornicotine	SS	110.00	390.00	ng/cig	8
Nitrosopyrrolidine	MS	2.60	52.00	ng/cig	5
Nitrosopyrrolidine	MS	1.50	29.00	ng/cig	7
Nitrosopyrrolidine	SS	204.00	612.00	ng/cig	5
Nitrosopyrrolidine	SS	28.00	143.00	ng/cig	7
Nitrosopyrrolidine	SS	80.00	500.00	ng/cig	8
Nitrosopyrrolidine, *N*-	MS	2.60	51.70	ng/cig	10
Nitrosopyrrolidine, *N*-	SS	204.00	387.00	ng/ciq	10
Octane (breath smokers)	IA	1.10		$\mu g/m^3$	30
Octane (breath, non-smokers)	IA	0.10		$\mu g/m^3$	30
Octane (homes, non-smokers)	IA	1.70	3.10	$\mu g/m^3$	30
Octane (homes, smokers)	IA	1.50	4.70	$\mu g/m^3$	30
Parvoline	MS	0.00	4.30	µg/cig	6
Parvoline	SS	10.00	145.00	µg/cig	6
Pentadien-4-olide, 2, 4-	MS	8.00	41.00	µg/cig	25
Pentadien-4-olide, 2, 4-	SS	71.00	256.00	µg/cig	25
Perylene	IA			Qual (ng/m^3)	1
Perylene	IA	11.00		ng/m^3	15
Perylene	IA	0.70	1.30	ng/m^3	20
Perylene	MS	9.00		ng/cig	14
Perylene	SS	39.00		ng/cig	14
Perylene control	IA	2.80	1.70	ng/m^3	20
Phenanthrene	IA			Qual (ng/m^3)	1
Phenanthrene	MS, P	74.80		ng/cig	16
Phenanthrene	MS, V	2.10		ng/cig	16
Phenanthrene	SS, P	2149.00		ng/cig	16
Phenanthrene	SS, V	248.00		ng/cig	16
Phenol	MS	79.00	136.00	µg/cig	23
Phenol	MS	77.00	139.00	µg/cig	25
Phenol	SS	69.00	241.00	µg/cig	23
Phenol	SS	157.00	289.00	µg/cig	25

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Phenol, 4-vinyl-	MS	18.00	45.00	µg/cig	25
Phenol, 4-vinyl-	SS	25.00	57.00	µg/cig	25
Phenols	IA	7.40	11.50	µg/m^3	20
Phenylacetic acid	MS	18.00	38.00	µg/cig	23
Phenylacetic acid	SS	11.00	30.00	µg/cig	23
Picoline, 3-	MS	12.00	22.00	µg/cig	25
Picoline, 3-	SS	90.00	166.00	µg/cig	25
Picoline, alpha-	MS	12.30	189.00	µg/cig	6
Picoline, alpha-	SS	128.00	1090.00	µg/cig	6
Pyran-4-one, 5, 6-diOH-3, 5-diOH-2-ME	MS	13.00	153.00	µg/cig	25
Pyran-4-one, 5, 6-diOH-3, 5-diOH-2-ME	SS	0.00	143.00	µg/cig	25
Pyrazine, 2, 3-dimethyl-	SS	0.00	50.00	µg/cig	6
Pyrazine, 2-methyl	MS	0.00	8.60	µg/cig	6
Pyrazine, 2-methyl-	SS	0.00	8.60	µg/cig	6
Pyrene	IA			Qual (ng/m^3)	1
Pyrene	IA	66.00		ng/m^3	15
Pyrene	IA	4.10	9.40	ng/m^3	20
Pyrene	MS	270.00		ng/cig	14
Pyrene	MS, P	43.00		ng/cig	16
Pyrene	MS, V	1.90		ng/cig	16
Pyrene	SS	1011.00		ng/cig	14
Pyrene	SS, P	466.00		ng/cig	16
Pyrene	SS, V	10.30		ng/cig	16
Pyrene control	IA	2.80	7.00	ng/m^3	20
Pyrene, 1-methyl-	IA			Qual (ng/m^3)	1
Pyrene, 2-methyl-	IA			Qual (ng/m^3)	1
Pyrene, 4-methyl-	IA			Qual (ng/m^3)	1
Pyridine	MS	32.40	648.00	µg/cig	6
Pyridine	MS	16.00	20.00	µg/cig	25
Pyridine	SS	336.00	3420.00	µg/cig	6
Pyridine	SS	187.00	262.00	µg/cig	25
Pyridine, 2-(3-pentyl)-	MS	0.00	1.50	µg/cig	6
Pyridine, 2-(3-pentyl)-	SS	0.00	143.00	µg/cig	6
Pyridine, 2-ethyl-	MS	2.60	35.00	µg/cig	6
Pyridine, 2-ethyl-	SS	2.60	35.00	µg/cig	6
Pyridine, 3-acetyl-	MS	3.80	6.40	µg/cig	24
Pyridine, 3-acetyl-	SS	9.00	11.00	µg/cig	24
Pyridine, 3-cyano-	SS	24.00	64.00	µg/cig	24
Pyridine, 3-cyano-	MS	2.40	4.20	µg/cig	24
Pyridine, 3-ethyl-	MS	4.00	6.00	µg/cig	25
Pyridine, 3-ethyl-	SS	71.00	960.00	µg/cig	6
Pyridine, 3-ethyl-	SS	21.00	36.00	µg/cig	25
Pyridine, 3-ethyl-4-methyl-	SS	6.40	34.00	µg/cig	6
Pyridine, 3-ethyl-4-methyl-	MS	0.00	1.50	µg/cig	6
Pyridine, 3-hydroxy-	MS	125.10	211.40	µg/cig	24
Pyridine, 3-hydroxy-	MS	90.00	119.00	µg/cig	25
Pyridine, 3-hydroxy-	SS	152.00	167.00	µg/cig	24
Pyridine, 3-hydroxy-	SS	157.00	191.00	µg/cig	25
Pyridine, 4-ethyl-	SS	27.00	379.00	µg/cig	6
Pyridine, 4-*t*-butyl-	MS	0.00	4.50	µg/cig	6
Pyridine, 4-*t*-butyl-	SS	17.00	287.00	µg/cig	26
Pyridine, methylvinyl-	MS	2.20	4.10	µg/cig	24
Pyridine, methylvinyl-	SS	12.00	19.00	µg/cig	23

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Pyrrole	MS	16.00	23.00	μg/cig	25
Pyrrole	SS	140.00	272.00	μg/cig	25
Styrene (breath, non-smokers)	IA	0.30		$\mu g/m^3$	30
Styrene (breath, smokers)	IA	1.10		$\mu g/m^3$	30
Styrene (homes, non-smokers)	IA	0.80	1.10	$\mu g/m^3$	30
Styrene (homes, smokers)	IA	1.10	2.20	$\mu g/m^3$	30
Succinic acid	MS	112.00	163.00	μg/cig	23
Succinic acid	SS	65.00	70.00	μg/cig	23
Succinic acid, methyl-	MS	4.00	31.00	μg/cig	23
Succinic acid, methyl-	SS	1.00	13.00	μg/cig	23
Tar radical sol in *t*-butylbenzene	MS			Qualitative	22
Tar radical sol in *t*-butylbenzene	SS			Qualitative	22
Thiocyanate (plasma, non-smoker)	IA	70.80		μmol/l	28
Thiocyanate (plasma, passive smoker)	IA	71.80		μmol/l	28
Thiocyanate (plasma, smoker)	IA	70.70		μmol/l	28
Thioethers (urine, non-smoker)	IA	6.00		mmol/ml	28
Thioethers (urine, passive)	IA	6.40		mmol/ml	28
Thioethers (urine, smoker)	IA	6.30		mmol/ml	28
Toluene	IA	0.04	1.04	mg/m^3	3
Valeric acid, 3-methyl-	MS	20.00	261.00	μg/cig	23
Valeric acid, 3-methyl	SS	20.00	384.00	μg/cig	23
Vinylphenol, *p*-	MS	21.00	51.00	μg/cig	23
Vinylphenol, *p*-	SS	21.00	45.00	μg/cig	23
Xylene, *m* + *p*- (breath, non-smokers)	IA	2.10		$\mu g/m^3$	30
Xylene, *m* + *p*- (breath, smokers)	IA	5.50		$\mu g/m^3$	30
Xylene, *o*- (breath, non-smokers)	IA	0.80		$\mu g/m^3$	30
Xylene, *m* + *p*- (homes, non-smokers)	IA	10.00	13.00	$\mu g/m^3$	30
Xylene, *m* + *p*- (homes, smokers)	IA	10.00	20.00	$\mu g/m^3$	30
Xylene, *o*- (breath, smokers)	IA	1.60		$\mu g/m^3$	30
Xylene, *o*- (homes, non-smokers)	IA	4.00	5.20	$\mu g/m^3$	30
Xylene, *o*- (homes, smokers)	IA	3.20	7.10	$\mu g/m^3$	30
Xylenol, 2, 6-	MS	8.00	16.00	μg/cig	23
Xylenol, 2, 6-	SS	8.00	20.00	μg/cig	23

[a] Listings are given in alphabetical order by compound and special conditions are noted in parentheses within the column labeled 'Compound'. The sample type is categorized in the second column as IA, indoor air; SS, sidestream smoke; MS, mainstream smoke; P, associated with particulate matter; and V, associated with volatile compounds.

[b] Reference numbers are as follows: 1, Alfheim and Ramdahl, 1984; 2, Ayer and Yeager, 1982; 3, Badre et al., 1978; 4, Brunnemann et al., 1976; 5, Brunnemann et al., 1977; 6, Brunnemann et al., 1978; 7, Brunnemann et al., 1980; 8, Brunnemann et al., 1983, 9, Brunnemann and Hoffmann, 1975; 10, Brunnemann and Hoffmann, 1978; 11, Elliott and Rowe, 1975; 12, Fischer et al., 1978; 13, Galuskinova, 1964; 14, Grimmer et al., 1977a; 15, Grimmer et al., 1977b; 16, Grimmer et al., 1987; 17, Harmsen and Effenberger, 1957; 18, Hinds and First, 1975; 19, Hugod et al. 1978; 20, Just et al., 1972; 21, Liu et al., 1974; 22, Pryor et al., 1983; 23, Sakuma et al., 1983; 24, Sakuma et al., 1984a; 25, Sakuma et al., 1984b; 26, Schmeltz et al., 1976; 27, Sexton et al., 1984; 28, Sorsa et al., 1985, 29, Stehlik et al., 1982; 30, Wallace and Pellizzari, 1986; and 31, Williams et al., 1985.

genotoxicity, one may calculate, in an additive fashion, the 'total genotoxic potential' of a cigarette. Comparative human exposure could then be calculated by knowing the total genotoxic potential of a cigarette, room air volume, and the air exchange rate. If one were to make a crude estimate of the bacterial mutagenicity of 'a cigarette' by using the upper concentration ranges from Table 1 and Salmonella potency range for the 10 bacterial mutagens (1 rev/100 mg to 1 rev/0.10 μg), the calculated mutagenic potency [μg of compound/cigarette × revertants/μg compound] of a cigarette would be approximately 0.5–45 revertants per cigarette. If all of the compounds in Table 2 except the known negatives were to be shown to be Salmonella mutagens, one could estimate the mutagenic activity level to be as high as 1500 revertants/cigarette. Obviously, this method of summing the total genotoxic activity for a 'typical cigarette' is most useful when the chemical and bioassay values are quantitatively accurate, when all genotoxicants have been identified and bioassayed properly, and when synergistic and/or antagonistic interactions do not interfere. Because one would not expect this list of bacterial mutagens to be inclusive of all the mutagens and because one cannot presently rule out all possible types of interactions, this type of calculation would estimate only the lower limits of mutagenicity as will be clearly demonstrated below.

Upon testing an acetone extract of SS particles, Löfroth et al. (1983) found the extract preferentially mutagenic in *S. typhimurium* TA98 in the presence of S9. The observed response using TA98 corresponded to 15 000 revertants per cigarette. Upon using a cigarette-smoking machine in a 15-m^3 room, Löfroth and Lazaridis (1986) calculated that their results represent the equivalent of 30 000 revertants per cigarette for MS and 10 000–20 000 revertants per cigarette for SS when using *S. typhimurium* TA98 with S9 activation. In 1987, Ling et al., using the same methods, observed for strain TA98 a range of 17 200–31 300 revertants per cigarette for SS organics tested using the plate incorporation protocol and a range of 36 000–118 300 revertants per cigarette using a micro-assay preincubation protocol. In addition, they observed significant activity using TA100 without

TABLE 2

THE GENOTOXICITY OF COMPOUNDS ASSOCIATED WITH ENVIRONMENTAL TOBACCO SMOKE

Compound	CAS Number	Bioassay results [a]	
Acetamide	60-35-5	CCC	+
		CT	+
		ST	NEG
Acetic acid	64-19-7	ST	NEG
Acrolein	107-02-8	ST	+
Anthracene	120-12-7	CCC	I
		CT	NEG
		CYC	NEG
		MNT	NEG
		ST	NEG
Benz[*a*]anthracene	56-55-3	CCC	+
		CT	+
		REC	NEG
		ST	+
		V79	+
Benzene	71-43-2	CCC	+
		CYI	+
		MNT	+
		SCE	NEG
		TRM	+
Benzo[*a*]pyrene	50-32-8	CCC	+
		CT	+
		CYC	NEG
		MDR	+
		MNT	+
		MNT	+
		MST	+
		SCE	+
		SRL	+
		ST	+
		V79	+
Benzo[*b*]fluoranthene	205-99-2	CCC	+
Benzo[*e*]pyrene	192-97-2	CCC	I
		CT	NEG
		REC	NEG
Benzo[*ghi*]perylene	191-24-2	ST	+
Benzoic acid	65-85-0	ST	NEG
Butyrolactone, gamma	96-48-0	ST	NEG
Cresol, *m*-, *o*-, and *p*-	95-48-7	ST	NEG
Dibenz[*a*, *j*]anthracene	224-41-9	ST	+
Formaldehyde	50-00-0	ASPD	+
		CCC	+
		MDR	+
		NEU	+
		REC	+
		SRL	+
		SRL	+
		ST	+
Hydrazine	302-01-2	CCC	+
		ST	+

TABLE 2 (continued)

Compound	CAS Number	Bioassay results [a]	
Hydroquinone	123-31-9	ALC	+
		ST	NEG
Indeno[1, 2, 3-*cd*]pyrene	193-39-5	CCC	+
Isoquinoline	119-65-3	ST	NEG
Limonene	5989-27-5	ST	NEG
Naphthalene	91-20-3	ST	NEG
Nicotine	54-11-5	NEU	NEG
		ST	NEG
Nitrosodiethylamine, *N'*-	55-18-5	CCC	+
		CT	+
		CYC	+
		L5	+
		MDR	+
		MST	NEG
		REC	+
		SCE	+
		ST	+
		V79	+
Nitrosodimethylamine, *N'*-	62-75-9	ARA	+
		CCC	+
		CT	+
		CYG	+
		CYG	NEG
		L5	+
		MNT	+/−
		MST	NEG
		NEU	+
		SCE	+
		SRL	+
		SRL	+
		ST	+
		V79	+
		YEA	+
Nitrosonornicotine	16543-55-8	CCC	+
Nitrosopyrrolidine	930-55-2	CCC	+
Perylene	198-55-0	SCE	NEG
Phenanthrene	85-01-8	ALC	+
		CCC	I
		CT	NEG
		CYC	NEG
		ST	NEG
Phenol	108-95-2	NEU	NEG
		ST	NEG
Pyrene	129-00-0	CCC	I
		CT	NEG
		CYC	NEG
		ST	NEG
		V79	NEG
Pyridine	110-86-1	SCE	+
		ST	NEG
Styrene	100-42-5	CCC	+
Toluene	108-88-3	SCE	NEG
		ST	NEG

[a] Bioassay information is extracted from Graedel et al., 1986. Abbreviations used for bioassay results are as follows: ALC, Allium cytogenetics assays; ARA, Arabidopsis mutagen assay; ASPH, Aspergillus mutagen assay; CCC, whole animal carcinogen assays; CT, cell transformation bioassays; CY, mammalian cytogenetic bioassays; L5, L5178Y mouse lymphoma assay; MDR, mammalian cell DNA repair assays; MNT, micronucleus assays; MST, mouse spot test; NEU, Neurospora assays; REC, DNA repair-deficient bacterial assays; SCE, sister-chromatid exchange assays; SRL, sex-linked recessive lethal assays in Drosophila; ST, Salmonella assays; TRM, Tradescantia mutagen assays; V79, V79 Chinese hamster mutation assays; and YEA, Yeast mutation tests. Results are recorded as +, positive; NEG, negative; and I, Indefinite.

activation. Ong et al. (1984) examined the mutagenicity of SS using *S. typhimurium* TA98W (an antibiotic-resistant strain of TA98) in an in situ impinger system. They stated that '... the concentration of cigarette smoke that could be detected for mutagenic activity was 0.0065 cigarette per ml.' Because the concentration of cigarette smoke in these experiments was equivalent to approximately 0.036 cigarettes/ml and the observed response was approximately 40 induced mutants/ml, they (Ong et al., 1984) observed 1111 [(40 rev/ml) · (1 ml/0.036 cig)] revertants per cigarette. However, if one assumes from their calculations that 0.0065 cigarettes would give a doubling (~ 20 induced revertants) of the spontaneous revertant number, the activity of SS is approximately 3080 [(20 rev/doubling) · (1 doubling/0.0065 cigarettes)] revertants per cigarette. The 3- to 10-fold difference between the results of the Löfroth et al. (1983) and Ong et al. (1984) is most likely due to differences in the collection and exposure systems used, although, once again, one cannot rule out the role of differences in chemical interactions. Löfroth et al. (1983) collected only particulate matter and tested the extracted organic material, whereas Ong et al. (1984), while attempting to test total SS, probably assayed primarily the

nonparticulate, semi-volatile organic material that would dissolve in the fluid medium. Although the methods of Löfroth et al. (1983) and Ong et al. (1984) did not identify the specific genotoxicants responsible for the biological activity, the direct bioassay of the emissions did provide a more accurate determination of the total mutagenic potential of a cigarette.

Knowing the amounts and bioassay activity of SS constituents or the total mutagenic activity of a 'typical cigarette' also does not necessarily indicate that one can accurately estimate human exposure to genotoxic (ETS) emissions. For both MS and SS, the amount of a specific material that is emitted from a cigarette ranges from sub-nanogram per cigarette levels to milligram per cigarette levels. The concentrations of constituents in smoke-impacted spaces range from ng/m^3 to mg/m^3; however, the calculation of ambient concentrations from cigarette emission rates is not always simple because ETS levels are functions of smoking rate, ventilation, sink rate, mixing, and volume of the space (National Research Council, 1986). Also, each individual compound may be removed from the ambient air at different rates due to these functions. For example, genotoxicant exposure levels can be approximated by measuring respirable particulate (RSP) levels and measuring the level of specific genotoxicants associated with the particles. RSP levels in a one-smoker residence can vary by 3 orders of magnitude from approximately 17 to 5000 $\mu g/m^3$ (National Research Council, 1986). Similarly, most IA concentrations (Table 1) for specific compounds span at least 2 orders of magnitude; however, the relative amounts of components with SS are often different from the relative amounts found in IA samples. It is also unlikely that all SS carcinogens have been identified. By collecting indoor air particulate matter and bioassaying the extracted organic matter using *S. typhimurium* TM677 in modified bioassay, Lewtas et al. (1987) demonstrated that particle-associated mutagenicity per cubic volume of air was greatest in the homes with cigarette smoking and correlated with the number of cigarettes smoked. The mutagenic activity per cigarette, however, could not be calculated in this study due to the presence of other potential sources of mutagens such as woodstoves, gas appliances, cooking, etc. Recently, Husgafvel-Pursiainen et al. (1986) demonstrated that indoor airborne particulate matter collected in 3 restaurants where smoking occurred gave up to 2370 revertants/m^3 air. On the basis of optical particle counting, they attributed the majority of the airborne particulate matter to cigarette smoking; however, they also did not estimate mutagenicity on a per cigarette basis. Husgafvel-Pursiainen et al. (1986), however, did show that the levels of polynuclear aromatic compounds roughly correlated with the mutagenic activity. Human exposure to tobacco-smoke genotoxicants, therefore, is highly variable and difficult to assess by evaluating individual components and/or equivalent bioassay activity levels emitted into ambient air.

In order to explore the use of bacterial bioassays in evaluating ETS, our laboratory has examined some of the alternative methods that can be used for evaluating ETS. The approaches used are briefly described in the Materials and methods section. As part of one study (Morin et al., 1987), filters used to collect SS particles were extracted with various solvents, solvent-exchanged to DMSO, and bioassayed. Depending upon the extraction conditions, tester strain, and activation conditions the revertants per cigarette ranged from 400 to 19000 (Fig. 1). Results using TA98 with S9 of ~19000 revertants/cigarette resemble data obtained by Löfroth et al. (1983).

Because the work of Ong et al. (1984) demonstrated that the semi-volatile and volatile components of SS may be mutagenic, we decided to assess the total mutagenic potential of both MS and SS. Using the sequential trapping train method described, we collected volatile, semi-volatile, and particulate-bound organics simultaneously from both MS and SS using separate trapping trains. Each of the fractions from both trains was bioassayed separately in the Salmonella mutagenicity assay. No mutagenicity was associated with the cold trap (volatile fraction) of either the MS or SS when evaluated as gases in the Tedlar dessicator system (Hughes et al., 1987). Table 3 summarizes the results for the other components of the trapping trains. Both the bubbler and sand trap samples were mutagenic for MS and SS; therefore, both the particulate and the semi-volatile components were mutagenic. The revertants/cigarette

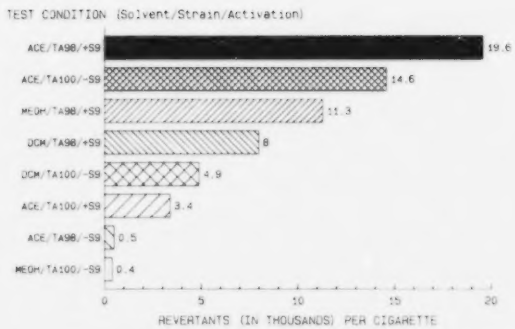

Fig. 1. *Salmonella typhimurium* mutagenicity of sidestream cigarette smoke when tested using various solvent extraction systems, strains of *S. typhimurium*, and activation conditions. Abbreviations are as follows: ACE, Acetone; DCM, Dichloromethane; MEOH, Methanol; TA98 and TA100 for respective strains of *S. typhimurium*; +S9, with exogenous activation; and −S9 without exogenous activation.

value of 35 200 is approximately twice the 19 000 and 15 000 values previously seen with particle-associated fractions; however, when the cigarette machine was not used to generate a puff mode sample and the cigarettes were allowed to smolder, this value was reduced to 23 250 revertants per cigarette. It has been demonstrated previously that the products produced during puffing and natural smoldering are somewhat different due to generated temperatures, the rate of mass transfer of oxygen to the tobacco source, properties of the cigarette paper, etc. (Baker, 1981, 1982). Therefore, it is reasonable that the mutagenicity of both MS and SS varies with the degree of puffing. The effect of burn conditions can also be noted by examining the revertants/μg of organic material values in Table 3. Compared on a per mass of organic material basis, the MS organics are 2–3 times as mutagenic as the SS organics. This demonstrates that the mainstream and sidestream combustion processes produce different relative amounts of bacterial mutagens. One also could speculate – from knowledge of the 2 types of combustion processes and differences in MS and SS chemistry – that the quantitative distribution of mutagens in MS and SS is different. Due to the mass of organic material produced, however, SS organics make a larger contribution to the total mutagenic activity of a cigarette. It is interesting to note that within our laboratory the response of benzo[*a*]pyrene (B[*a*]P) in *Salmonella typhimurium* TA98 with exogenous activation is approximately 20 revertants/μg; therefore, one can calculate that total cigarette bacterial mutagenicity is equivalent to ~2.6 mg of B[*a*]P. Approximately 70% (61 600/86 300) of the total mutagenicity is associated with the sand trap (particulate) samples. This proportion also is approximately the same for the MS and SS samples. Overall, the SS sample accounted for approximately 60% of the total mutagenicity on a per cigarette basis.

The best way presently available to assess human exposure to genotoxic ETS compounds is by the analysis of the fluids and tissues of exposed individuals. Exposure of target tissues and sites to genotoxic constituents of ETS depends on several factors, including the number of cigarettes smoked

TABLE 3

THE *SALMONELLA TYPHIMURIUM* TA98 MUTAGENICITY OF MAINSTREAM AND SIDESTREAM TOBACCO SMOKE COLLECTED IN A PUFF MODE

Fraction	Ethanol bubbler		Sand trap		Bubbler + sand
	Rev/μg [a]	Rev/cig [b]	Rev/μg	Rev/cig	Rev/cig
Main	8.1	7557	4.0	26 400	33 957
Side	2.7	17 099	1.6	35 200 [c]	52 299
Total		24 656		61 600	86 256

[a] *S. typhimurium* TA98 mean revertants per plate per μg of organic material collected using a preincubation bioassay. Determined using a linear regression model (Myers et al., 1981)

[b] *S. typhimurium* TA98 revertants per cigarette calculated from (Rev/μg)×(μg of fraction/cigarette).

[c] When puff mode was not used and the cigarettes were allowed to smolder, this value was 23 253 rev/cig.

in an enclosed area, the size and nature of the area, the degree of room ventilation, breathing rates and volume, absorption of the genotoxicants, body distribution and excretion, and metabolism. The uptake of individual agents from ETS can be determined using chemical methods. For example, one can measure products such as thiocyanate, carboxyhemoglobin, nicotine and cotinine in physiological fluids. However, some of these (e.g., thiocyanate due to HCN exposure and carboxyhemoglobin from CO exposure) do not originate exclusively from SS (National Research Council, 1986). The quantitative aspects of other chemical and biochemical markers such as cotinine, hydroxyproline, *N*-nitrosoproline, and aromatic amines are still somewhat questionable (National Research Council, 1986). In addition, when one bioassays an agent individually, it may not be known whether or not different genotoxic compounds are absorbed, distributed, metabolized, and excreted at the same rate as each other. The use of a noncompound-specific measurement of exposure to genotoxicants, therefore, would be very advantageous. The monitoring of nonsmokers' urine for mutagenic potential using bacterial assays provides a possible means of evaluating in vivo exposure to ETS genotoxicants. In 1977, Yamasaki and Ames reported the presence of bacterial mutagens in the urine of smokers. As noted by IARC (1986), a number of studies have confirmed the finding of Yamasaki and Ames; however, there is a wide variation in the results of these studies. Much of this variation may be due to dietary factors (Sasson et al., 1985). The studies that have examined the urine mutagenicity of passive smokers (Putzrath et al., 1981; Bos et al., 1983; Sorsa et al., 1985) demonstrated increased mutagenicity in adult passive smokers. These studies generally examined small numbers of people and did not control for dietary factors; therefore, the results might be considered somewhat ambiguous. In spite of the shortcomings, these studies did support the use of bacterial bioassays as a screening tool for human exposure to ETS genotoxicants; however, more effort is needed in improving and standardizing the methods and in creating the proper controls for other environmental factors such as diet.

In summary, our studies support previous studies that demonstrated that ETS particle-bound organic material is mutagenic. In addition, our studies demonstrated that some semi-volatile and volatile components were mutagenic; however, the highly volatile compounds (for both MS and SS) collected in the third-stage liquid nitrogen cold trap were not mutagenic. Within these studies, the total mutagenicity was divided among 4 fractions approximately as follows: SS sand trap (particulate) fraction, 40%; MS sand trap (particulate) fraction, 30%; SS solvent (semi-volatile) fraction, 20%; and MS solvent (semi-volatile) trap fraction, 10%. Results also gave an indication that the frequency with which a cigarette is puffed affects the total amount of mutagenic material produced. Although these studies illustrate the usefulness of bacterial mutagenicity bioassays for characterizing ETS, there are also other uses (e.g., identifying specific genotoxicants) for which bacterial assays will find great utility.

Acknowledgements

The technical assistance of Linda Monteith, Debra Simmons, Jack Callahan, Ken Davis, and Jeff Keever is gratefully appreciated. The authors wish to thank Dr. Göran Löfroth and Ms Virginia Houk for their careful review and suggestions for the manuscript. The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

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